REMARKS

Rejection Under 35 USC 103

Claims 1, 2, 4-6, 8, 9, and 11 have been rejected under 35 USC 103(a) as being unpatentable over Kagaya (EP 0727653) in view of Grow et al. (EP 0281251). More specifically, the Patent Office states:

> Kagaya teaches a device and method for sampling feces. The device includes a main container (13) which holds a sample collecting stick (15) with a brush portion (18) on the end. The brush is submerged in a liquid (12) that is contained in the lower portion of the main container (13)..."feces can be collected by rolling the brush over the feces."...Kagaya, then, teaches the collection of a fecal sample and the placing of that sample into a collection container where it is mixed with a liquid...Grow et al. teach methods for testing samples derived from fecal material...It would have been obvious to one of ordinary skill in the art to combine the assay steps of Grow with the collection steps of Kagaya.

In response, Applicant respectfully traverses this rejection. A fundamental difference between the method for collecting a faecal sample which is defined in the claims of the present application, and the assay kit of this invention, lies in an essential technical feature of the present invention which is the use of a sampling device such as a brush "having flexible or semi-flexible bristles", which enables a sample of fluid surrounding the faecal material to be obtained, optionally after "brushing" the surface of the material, as described at page 6 lines 24-29 and page 7 lines 20-27 of the present specification. Accordingly, whilst the brush or brush-like device defined in the present claims may be used to "paint" the surface of the stool so as to displace any blood on the surface of the stool into the water surrounding the stool (as described at page 6 lines 23-24), the sample which is contained is not a sample collected by directly contacting the brush with the faeces (as in Kagaya), rather it is a sample of the fluid surrounding the faecal material. Thus, the sampling device of the present invention in which the brush or brush-like device has flexible or semi-flexible bristles, collects a fluid sample within the bristles of the sampling device in a semi-quantitative manner, as the amount of fluid held within the interstitial spaces of the bristles of the sampling device will be a reasonably constant amount for any particular size and configuration of the sampling device (see page 7 lines 13-18). The essential technical feature of the present

invention is the use of the sampling device with flexible or semi-flexible bristles which collects a fluid sample in a semi-quantitative manner, preferably, but not essentially, after use of the sampling device to "paint" the surface of the faecal material.

Clearly, in the device taught by Kagaya, the "brush (18)" taught by Kagaya does not have "flexible or semi-flexible bristles" such that a fluid sample collected within the bristles of the sampling device is collected in a semi-quantitative manner within the interstitial spaces of the bristles of the sampling device as described in the present application. In fact, the brush portion (18) of the device shown in Figures 1 and 2 of Kagaya is described as having a plurality of brush hairs (20) which are fixed "so that external shape formed by connecting tips of brush hairs is approximately in cylindrical shape" (see column 3 lines 41-46). As acknowledged by the Examiner, in the device taught by Kagaya, the brush portion (18) is designed so that "faeces can be sampled by rolling the brush over the faeces" (see column 3 lines 47-49). Thus, the device described by Kagaya is a device for collecting a sample of faeces and not a device for collecting a fluid sample in a semi-quantitative manner, as described in the present application. Accordingly, Kagaya does not disclose or teach the use of a brush or brush-like device as recited in the present claims in which a fluid sample is collected within the flexible or semi-flexible bristles of the device. Furthermore, Grow *et al.* does not contain any disclosure or teaching which, if Kagaya was to be read in view of this document, would provide any disclosure or teaching of features which are not taught by Kagaya.

The device taught by Kagaya is a device which is designed for stool sampling and would not work effectively for collection of a sample of fluid which has been contacted with faecal material as recited in the present claims. Applicant respectively submits that sampling a fluid such as water around the stool, the brush or brush-like device of the present invention obtains a sample that is representative of the whole stool, as blood from the whole stool is released into the water. In contrast, the stool sampling device taught by Kagaya samples only a small part of the stool and the sample is therefore not representative of the whole stool. This is a significant disadvantage as blood released in small amounts from early neoplasia may only be striped or spotted on the stool and may be missed as taught by Kagaya. Such devices, therefore, require multiple samples to be taken. The effectiveness of the use of a brush or brush-like device having flexible or semi-flexible bristles as taught in the present

application is surprising insofar as it provides equivalent sensitivity with only two fluid samples when compared with a comparative immunochemical test using six stool samples from three stools. This comparison is described in the enclosed paper by Young *et al.* (2003) which discloses a pre-screening evaluation of the brush-sampling technique of the present invention (referred to as InSureTM) relative to a traditional spatula-sampling test which confirms that the use of a brush-sampling method in accordance with the present invention is as sensitive and specific as the traditional spatula-sampling method, and is highly preferred in use.

To more specifically define the invention of Claim 9 over the prior art, Applicant has amended Claim 9 to include the phrase "wherein a fluid sample may be collected within the bristles of the brush or brush-like device". Basis for this amendment may be found in claims 1 and 6, and elsewhere throughout the present specification.

Additionally, Claims 3 and 10 have been rejected under 35 USC 103(a) as being unpatentable over Kagaya (EP 0727653) in view of Grow et al. (EP 0281251) and further in view of Hori et al. (US Pat. No. 5,460,781). More specifically, the Patent Office states:

> Kagaya and Grow...teach every element of claims 3 and 10 except for the specific bristle length. Hori et al. teaches a hemoglobin sampler for use with stool samples for clinical tests. The device securely samples occult hemoglobin with water content...It would be obvious ...to combine the fiber dimensions from the sampler of Hori with the combined brush teachings of Kagaya and Grow...

In response, Applicant respectfully submits that the device taught by Hori *et al.* is not a brush or brush-like device having flexible or semi-flexible bristles wherein a sample of fluid may be collected in a semi-quantitative manner within the bristles of the brush or brush-like device. Accordingly, Hori *et al.* does not contain any disclosure or teaching of the features which are not taught by Kagaya and Grow *et al.*

Additionally, Claims 8 has been rejected under 35 USC 103(a) as being unpatentable over Kagaya (EP 0727653) in view of Grow et al. (EP 0281251) and further in view of Schreiber et al. (US Pat. No. 5,264,181). More specifically, the Patent Office states:

> Schreiber teaches the detection of occult blood fecal matter using a guaiac test...It would have been obvious...to perform the guaiac test from Schreiber to determine the presence of blood in a fecal sample. One would perform the

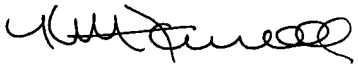
guaiac test as an additional check for blood material in the fecal sample in combination with the assay materials used in Grow.

In response, Applicant respectfully submits that Schreiber does not teach the use of a brush or brush-like device having flexible or semi-flexible bristles in the collection of a fluid sample in a semi-quantitative manner within the bristles of the brush or brush-like device. Accordingly, Schreiber does not contain any disclosure or teaching of features of the present invention which Kagaya and Grow *et al.* fail to teach. In conclusion, Applicant respectfully submits that the present invention provides a simple and effective method of collecting a fluid sample associated with faecal material in a semi-quantitative manner, and which is both novel and non-obvious in the light of the prior art documents cited.

Summary

In light of the above amendment, consideration of the subject patent application is respectfully requested. Any deficiency or overpayment should be charged or credited to Deposit Account No. 500282.

Respectfully submitted,



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ORIGINAL PAPER

Prescreening evaluation of a brush-based faecal immunochemical test for haemoglobin

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Objectives: To undertake a prescreening evaluation of a new brush-based faecal immunochemical test for haemoglobin, relative to a traditional spatula-sampling immunochemical test.

Methods: *Setting*: Patients aged between 24 and 90 years, scheduled to undergo diagnostic colonoscopy in two major urban hospitals, for a range of clinical indications. *Design:* Patients sampled three stools using a spatula for the reference FlexSure® OBT test and two stools using a brush for the InSure™ test; order of sampling was randomised. Faecal haemoglobin was quantified by a modified InSure in a subset of patients to determine whether brush-sampling allowed discrimination between groups. *Main outcome measures:* Sensitivity for cancer or adenoma; false-positive rate in normals. Faecal haemoglobin levels. Preference for sampling method.

Results: InSure and FlexSure OBT did not differ in their sensitivities for cancer (27/36, 75% *vs* 29/36, 80.5%, respectively), adenomas ≥10 mm (12/29, 41.4% *vs* 13/29, 44.8%) or adenomas <10 mm (each 8/56, 14.3%). Likewise, false-positive rates in normals were similar: 4/179 (2.2%) and 5/179 (2.8%) respectively (specificities of 97.8% and 97.2%, respectively). Levels of faecal haemoglobin were highest in those with cancers; those with adenomas had intermediate levels which were also significantly higher than those in normals. The brush sampling method was preferred by 38/46 (82.6%), while 4/46 (8.7%) preferred the spatula ($p<0.00001$).

Conclusions: InSure is as sensitive and specific as FlexSure OBT for faecal haemoglobin. The novel stool-sampling method of InSure allows discrimination between normals and classes of neoplasia, and is highly preferred. The brush-sampling faecal immunochemical test InSure should now be evaluated in a screening population.

INTRODUCTION

The type of faecal occult blood test (FOBT) used in the randomised controlled trials of screening[1–4] for colorectal cancer has previously been the guaiac-based test Hemoccult®. Guaiac detects the peroxidase activity of haem (and other peroxidases in the stool) but may be subject to false-positive and false-negative results brought about by certain dietary components, vitamin supplements and drugs.[5–8] It also uses a wooden spatula for sampling faeces.[9] Recently, new technologies for sampling faeces and detecting occult blood have become available.[10] These are based on antibody detection of blood and use a range of technological means, including tagged red cell agglutination, latex agglutination or immunocapture technologies where reagents diffuse across a membrane, to provide the result. If these new technology FOBTs simplify testing and so increase participation, they should be used for population screening.

Human globin can now be detected using an analytically specific antibody.[11,12] These faecal immunochemical tests for globin provide several advantages.[13,14] They are unaffected by diet or drugs,[6,8] are selective for occult bleeding of colorectal origin and do not detect occult gastric bleeding.[15,16] As a class of test for faecal occult blood, immunochemical tests also provide improved sensitivity for colorectal cancer without major compromise of specificity[17] when compared with guaiac tests. Where the sensitivity of guaiac tests was increased, specificity was adversely affected unless interfering dietary factors and drugs were restricted.[1,5,18–20] However, such restrictions created a significant barrier to participation in screening.[20–24]

The method for sampling faeces has also been varied in an attempt to overcome aversion to sampling faeces.[25–28] The spatula-sampling method is not liked, and its accuracy is better when used to sample a stool kept clear of toilet bowl water.[29] Newer immunochemical tests use non-spatula sampling methods to simplify sampling, using devices such as probes, spoons or brushes.[30–32] One of these, the brush-sampling method, does not require the stool to be kept clear of toilet bowl water. It might therefore be more acceptable to screenees who are relatively averse to handling faeces. While the immunochemical class of test has been well evaluated in screening settings,[10] the brush-sampling method has not and requires comparative evaluation with a spatula-sampling method before more extensive testing in population screening.[33]

Thus, we undertook a prescreening evaluation of a new brush-sampling test (InSure™) relative to a traditional spatula-sampling test (FlexSure® OBT), in patients scheduled to undergo diagnostic colonoscopy. Patients sampled three stools for the FlexSure OBT test and two stools for the InSure test; test order was randomised. In addition, we also adapted the InSure test to a quantitative format, to determine whether it is able to discriminate between normal subjects and those with different stages of neoplasia. We reasoned that for a sampling method to be valid, it should show such discrimination.

MATERIALS AND METHODS

Study population

Patients seen at the two institutions were considered for inclusion in the study if they were scheduled for diagnostic

or surveillance colonoscopy in a high-risk program, during the period January 1999 to August 2001. To be eligible, they had to be capable of following instructions, able to sample stools, and not be taking any drugs able to cause gastrointestinal bleeding. They were ineligible if they had a known colonic disorder likely to cause bleeding (except neoplasia) or had previously undergone colorectal surgery. The goal was to study at least 100 patients with neoplasia, one-third of whom had cancer; this was reached when 524 consenting patients had met these conditions

Faecal occcult blood tests

Invitees were asked to complete the screening tests according to each manufacturer's instructions and to return samples to the study site at, or prior to, the time of colonoscopy. No dietary or drug restrictions were recommended. The order for faecal-sampling by each test type was specified after prior randomisation.

FlexSure OBT (Beckman Coulter Inc., Palo Alto CA, USA) is an immunochemical test with analytical characteristics comparable with those of Immudia-Hem Sp (Fujirebio Inc., Tokyo, Japan) and the now discontinued HemeSelect (Beckman Coulter Inc.)[34] (also see product inserts). Invitees were asked to sample each of three stools (one card per stool), keeping the stool clear of toilet bowl water and using a spatula similar to that for Hemoccult. This was used as the reference test for comparison as the US Food and Drug Authority (FDA) had approved FlexSure OBT as an appropriate comparator for new immunochemical tests on the basis of information provided to it by the manufacturers of FlexSure OBT.

InSure (Enterix Inc., Portland ME, USA) is a new immunochemical test approved by the FDA. Like FlexSure OBT, it uses membrane technology and immunolabelled colloidal gold to detect haemoglobin, and diet and drug restrictions are therefore unnecessary. It uses a different approach to faeces sampling, and requires sampling from two rather than three stools. The invitee is asked to sample the stool by briefly brushing its surface while it is immersed in toilet bowl water. The brush retains an approximately constant volume that is transferred by dabbing it onto one of two windows of the sample card. The second stool is separately sampled onto the other window.

Development and follow-up

Tests were developed by the authors (SC, BC, BB and MS) according to manufacturers' directions, after instruction from the manufacturers and proficiency testing. Positive controls were included for each test batch.

If any stool sample for a given test returned a positive reaction, the overall result for that test was considered positive. Diagnosis was ascertained from colonoscopy and pathology reports. Colonoscopists and pathologists remained unaware of the results of the FOBTs and they reported their findings without reference to the authors.

When more than one adenoma was present, adenoma location was defined as the site of the largest lesion.

For results to be successfully analysed, faecal sampling must have been performed within the eight-week period before the colonoscopy or between colonoscopy and surgery, and diagnostic evaluation of the colon must have been considered complete by the proceduralist. In addition, stool sampling must have been performed correctly and kits must have been stored and developed within the time limits set by each manufacturer for each test (i.e. from time of sampling faeces to development). If a sample card was not returned in time to meet this limit, the participant was excluded from the study.

Quantification mode

To verify the sampling method for InSure, a quantitative imaging methodology was used to measure faecal haemoglobin in a random subset of cases recruited between June 1999 and June 2000. Colour images of the InSure test strip window were captured using an Elmo charged coupled device digital camera (Plainview, NY, USA) and analysed with proprietary software routines developed in partnership with CSIRO Mathematics and Statistics Division (Sydney NSW, Australia). To determine a relative faecal haemoglobin level, each test strip was scored by measuring the intensity ratio (IR) of the haemoglobin test line signal relative to an internal control line signal, where a value of 1.0 indicated a test with an intensity equal to the control line. Intensity ratio values were log-normalised and compared between those with cancer, adenoma, or normal colonoscopy by t-test using Excel 97 software (Microsoft Corp., USA).

Survey of test preference

Test preference was assessed in a subset of 46 subjects randomly selected for a structured telephone survey. All were asked by telephone, within six weeks of performing sampling, which test they preferred and why.

Outcome measures and analyses

Primary analyses were conducted on data from those participants in whom neoplasia was found or in whom the colon/rectum was normal. A normal colon/rectum was defined as absence of benign or malignant disease. Those found subsequently to have benign colorectal disorders at colonoscopy were divided into those with haemorrhoids, those hyperplastic polyps and no other pathology, those with diverticular disease and no other pathology, and those with other benign disease (including multiple diagnoses).

Primary aim 1

To determine and compare test sensitivities for cancer, test positivity rates in those with cancer were compared by paired 2 × 2 analysis with calculation of 95% confidence intervals (CI) of the difference.[35]

Primary aim 2

To determine and compare test sensitivities for adenomas (sizes <10 mm or ≥10 mm, test positivity rates in those with neoplasia were compared by paired 2 × 2 analysis with calculation of 95% CI of the difference.[35]

Primary aim 3

Specificity was calculated for each test from 1-false positive rate in those with a normal colon and rectum and compared by paired 2 × 2 analysis with calculation of 95% CI of the difference.[35]

Secondary aims

Test positivity rates were determined and compared by χ^2 test (using SPSS for Windows release 10.0.5 software [SPSS Inc., 1999] Chicago, USA) in those with benign pathologies, specifically haemorrhoids, hyperplastic polyps or diverticular disease. Test preference rates were determined and compared by the same method.

Ethical approval was received in December 1998 from the Research and Ethics Committees of the Repatriation General Hospital Daw Park (number 42/98), and from the Clinical Research and Ethics Committee of The Royal Melbourne Hospital Research Foundation (number 2000.026). The research ethical guidelines of the National Health and Medical Research Council of Australia were followed.

RESULTS

Recruitment

Of those consenting to the study, 443 met all the requirements of the study, including complete colonoscopy. At colonoscopy, the following pathological outcomes were found: cancer, 36; adenoma, 85; normal examination, 179; haemorrhoids, 20; diverticular disease, 63; hyperplastic polyps, 15; and other miscellaneous benign pathologies, 45. The demographics of these participants are described in Table 1.

Table 1 Demographic characteristics of patients in the various diagnostic categories

Colonic diagnosis	Age (years) (median, range)	Gender (M:F)
Normal	59 (28–90)	76:103
Cancer	67 (45–81)	26:10
Adenoma ≥10 mm	67 (31–83)	19:10
Adenoma <10mm	68 (24–86)	35:21
Haemorrhoids	64 (34–84)	10:10
Hyperplastic polyps	57 (50–79)	10:5
Diverticular disease	68 (38–84)	30:33

M, male; F, female.

Sensitivity

Table 2 shows the results for each test in each diagnostic category. Table 3 shows the results of paired comparisons.

Table 2 Test positivity rates by colonoscopic diagnosis

	Number positive/total cases for diagnosis	
Cases for diagnosis	InSure	FlexSure OBT
Normal	4/179 (2.2%)	5/179 (2.8%)
Haemorrhoids	3/20 (15%)	6/20 (30%)
Diverticular disease	8/63 (12.7%)	4/63 (6.3%)
Hyperplastic polyps	0/15 (0%)	0/15 (0%)
Cancer, all	27/36 (75%)	29/36 (80.5%)
Proximal	6/8 (75%)	6/8 (75%)
Stage A or B	16/21 (76.2%)	16/21 (76.2%)
Adenomas ≥10 mm, all	12/29 (41.4%)	13/29 (44.8%)
Villous or tubulovillous	8/15 (53.3%)	8/15 (53.3%)
Adenomas <10 mm	8/56 (14.3%)	8/56 (14.3%)

Sensitivity for cancer

For cancer, the sensitivities of InSure, at 75%, and of FlexSure OBT, at 80.5% (Table 2), were not significantly different (Table 3).

Subcategorising cancers by stage, there was no difference between tests for cancers of stage A or B; sensitivities of 76.2% were found for each. Likewise, subdividing cancers by location, proximal or distal to the splenic flexure, there were no differences in sensitivity; for proximal cancers, sensitivities were 75% for each test; and for distal cancers, sensitivities were 75% for InSure and 82.1% for FlexSure OBT.

Table 3 Concordance between paired results for each diagnostic category

		FlexSure OBT	
Normals		Positive	Negative
InSure	Positive	0	4
	Negative	5	170

Difference 0.6%, 95% confidence intervals (CI) of difference −2.7% to 3.8%.

		FlexSure OBT	
Haemorrhoids		Positive	Negative
InSure	Positive	2	1
	Negative	4	13

Difference 15%, 95%CI of difference -5.9% to 35.9%.

		FlexSure OBT	
Diverticular disease	Positive	Negative	
InSure	Positive	3	5
	Negative	1	54

Difference 6.3%, 95%CI of difference −1.1% to 13.8%.

		FlexSure OBT	
Cancers, all		Positive	Negative
InSure	Positive	25	2
	Negative	4	5

Difference 5.6%, 95%CI of difference −7.7% to 18.8%.

		FlexSure OBT	
Adenomas ≥10 mm		Positive	Negative
InSure	Positive	9	3
	Negative	4	13

Difference 3.4%, 95%CI of difference −14.4% to 21.3%.

		FlexSure OBT	
Adenomas <10 mm		Positive	Negative
InSure	Positive	4	4
	Negative	4	44

Difference, zero.

Sensitivity for adenomas ≥10 mm

For these larger adenomas, sensitivities of InSure, at 41.4%, and of FlexSure OBT, at 44.8% (see Table 2), were not significantly different (Table 3). Subcategorising adenomas by degree of villous change (see Table 2) did not reveal any significant differences. Sensitivity for proximally located larger adenomas was equal (Table 2).

Sensitivity for adenomas <10 mm

Both tests returned a sensitivity of 14.3% for adenomas <10 mm (Table 2).

Specificity

False-positive rates in normals were 4/179 (2.2%) and 5/179 (2.8%) for InSure and FlexSure OBT, respectively (specificities were 97.8% and 97.2%, respectively) (Table 2). The results were not significantly different.

Test positivity rates in presence of benign pathologies

Positivity rates for InSure and FlexSure OBT are shown in Table 2 for benign conditions, i.e. haemorrhoids, diverticular

disease and hyperplastic polyps. For FlexSure OBT, the positivity rates were significantly higher in those with haemorrhoids than in normals (χ^2=20.6, p<0.0001) but not in those with diverticular disease compared with normals (χ^2=0.80, p=0.37). For InSure, the positivity rates were significantly higher for both those with haemorrhoids (χ^2=5.3, p=0.022) and diverticular disease (χ^2=8.72, p=0.004) than in normals.

Concordance in test results

Table 3 shows the concordance between results for all diagnostic categories. The highest degree of concordance for positive results was seen for cancers (25/31, 80.6%); progressively lower rates were seen in those with large adenomas (9/16, 56.3%), small adenomas (4/12, 33.3%) or no pathology.

Validity of sampling method

Figure 1 shows quantitative measures of faecal haemoglobin in subjects with normal colon, adenomas or cancer. Respective mean intensity ratios are 0.011±0.002, 0.095±0.040, and 0.295±0.049 standard error of the mean. It can be seen that those with cancers had the highest levels of bleeding (p=0.00003 *vs* normals and p=0.002 *vs* adenomas). Those with adenomas had intermediate levels, significantly higher than normals (p=0.039) although showing overlap with them, and significantly lower than those with cancer.

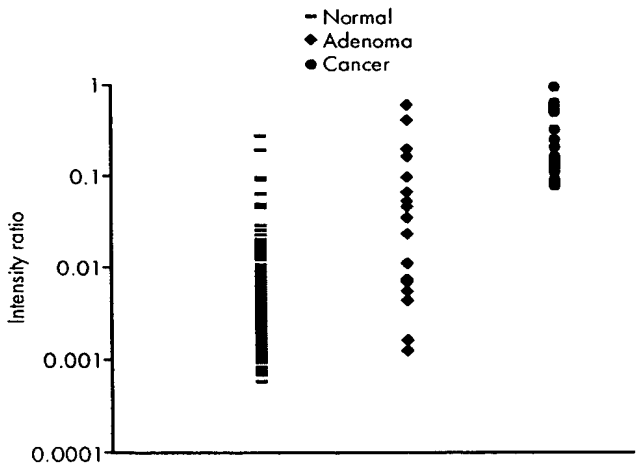

Figure 1 Quantification of haemoglobin in subsets of patients within selected diagnostic categories: normal colon and rectum, adenomas or cancer. Faecal haemoglobin levels are shown as arbitrary units. An intensity ratio of 0.03 represents the 95th percentile for normals and corresponds to approximately 750 µg of haemoglobin per litre of toilet bowl water.

Test preference

Thirty-eight of 46 subjects preferred InSure, four preferred FlexSure OBT and four had no preference (χ^2=22.9, p<0.00001). The reasons given were always that the nominated test was easier, except in two subjects: one considered the brush method to be more hygienic, and the other felt more certain that a satisfactory sample had been obtained using the spatula.

DISCUSSION

The demonstrated impact on mortality of screening using guaiac FOBTs[1–4] is limited for two main reasons: test sensitivity and willingness of invitees to perform the test, i.e. to participate. In UK and Danish trials of biennial screening with Hemoccult, mortality was reduced on an intention-to-screen basis by 15% and 18%, respectively, with participation rates of a little over 50%.[2,3] The sensitivity of Hemoccult in these studies was reported as 65.6%[36] and 46.6%,[37] respectively. Improving either or both would enhance the value of screening at the population level.[33]

Immunochemical tests, especially if combined with a more acceptable faecal sampling method, may provide these improvements. However, prior to formal evaluation in population studies, it is necessary to undertake prescreening validation in a clinical setting that demonstrates at least equivalence with existing technologies.[38]

By studying a population in two major urban hospitals, scheduled to undergo diagnostic colonoscopy for a range of clinical indications, we were able to show comparable sensitivity and specificity for the brush-sampling Insure when compared with the FDA-approved reference test FlexSure OBT.

This type of prescreening evaluation has been conducted for several pre-existing tests for blood in faeces. These have demonstrated differences between several guaiac tests,[19] between guaiac and immunochemical tests,[19,34,39,40] and between several immunochemical tests.[41,42] This type of study uses clinical populations in which colonoscopy is indicated because of symptoms; cancers are thus more likely to be relatively advanced (stage C or D). Such studies might therefore over-estimate sensitivity for the stage of cancer prevalent in screening populations, as earlier stage cancers bleed less.[38] Nonetheless, they are useful for testing equivalence to existing tests in a setting where there are no ethical issues arising from poor specificity or sensitivity.[38] In similar clinical settings, the sensitivity of FlexSure OBT for cancer has been observed to be 70–85%,[34,39] which compares well with this study.

Both tests returned similar sensitivities for adenomas. The lower sensitivity of both FlexSure OBT and Insure for adenomas compared with cancers is consistent with results from all studies in which FOBTs have been used to address this question.[10] In the present study, we observed an acceptable level of sensitivity (over 40%) of both tests for larger adenomas. This is in keeping with an earlier study which found immunochemical tests to be more sensitive than guaiac tests for adenomas,[19] although this has not been a consistent observation with immunochemical tests.[34] The poor sensitivity of both tests for small adenomas in our study is again in keeping with previous studies.[10] The importance of detection of small adenomas is unclear,[43] and no study has directly demonstrated that their removal prevents cancer.

Because the two immunochemical tests were compared in a paired setting, we were able to test for discordant results (Table 3). For cancers, 17% of positives were discordant compared with 24% for large adenomas. One would expect some discordance, since colorectal neoplasms are known to bleed intermittently[44] and the different sampling methods required participants to sample with each test on different days. These low rates of discordance, especially for cancers, suggest that intermittency of bleeding might not be a major issue for immunochemical tests. The Insure development card did not allow us to calculate day-by-day sensitivity relative to FlexSure OBT. Although the Insure test samples

only two stools, it provided the same sensitivity as FlexSure OBT.

Immunochemical tests have specificity advantages over guaiac tests because they are unaffected by diet or drugs.[6,8] With guaiac tests, high intake of vitamin C, [7] administration of non-steroidal anti-inflammatory drugs (NSAIDs),[8] ingestion of plant peroxidases, or ingestion of haem from dietary blood and myoglobin (such as in red meats)[5] can affect accuracy. With immunochemical tests, there is no need to change diet or medication.[8,16,19] Indeed, the specificity of both tests in this study was excellent and met desirable standards easily for tests for colorectal cancer screening.[10]

The brush-sampling method is novel and appears to go against current recommendations that stools be sampled for blood from the surface while kept clear of toilet bowl water.[33] Nonetheless, the method is clearly valid since the quantitative results show good discrimination between normals and those with cancer.

Aversion to manipulating faeces and inconvenience are some of the barriers to screening.[45] The simplification of sampling, either by sampling fewer stools or by making the sampling method easier or more acceptable, should address both inconvenience and faecal aversion. We observed an overwhelming preference for the brush-sampling method. This seems likely to present an advantage at the population level by improving participation.

There are possible disadvantages of the sampling method for Insure, such as urine contamination and interference by toilet bowl additives, but the manufacturer's instructions demonstrate that these should have only minimal effect. It is also possible that by sampling toilet bowl water around the stool, positives might be more likely in the presence of haemorrhoidal bleeding. However, there was no significant difference between the positivity rates for the two tests, demonstrating that the brush-sampling method does not lead to an excess of positives in the presence of haemorrhoids. Both immunochemical tests gave more positives in those with haemorrhoids or diverticular disease than were seen in those with a normal colon and rectum. Technically, these are false-positives when screening for colorectal cancer. Differences in prevalence of these benign disorders might account for, or at least contribute to, differing false-positive rates between populations.[10]

CONCLUSION

In this pre-screening evaluation, the brush-sampling immunochemical technology of the InSure test is shown to be as sensitive and specific as is the FlexSure OBT for faecal globin. The novel stool-sampling method is valid, based on its ability to discriminate between normals and classes of neoplasia. Our observations suggest that, in the context of population screening for colorectal cancer, individuals will be more willing to perform a brush-based faecal immunochemical test than one utilising the traditional spatula method for specimen collection. If so, this should lead to better detection of neoplasia in population screening.

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